

# Outbreak of Listeriosis among Mexican Immigrants as a Result of Consumption of Illicitly Produced Mexican-Style Cheese

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**Background.** In 2000, an outbreak of listeriosis among Hispanic persons was identified in Winston-Salem, North Carolina. The objectives of the present study were to identify the source of, strains associated with, and risk factors for *Listeria monocytogenes* infection for patients affected by the outbreak.

**Methods.** Microbiological, case-control, and environmental investigations were conducted. Participants in the case-control study were case patients who became infected with *L. monocytogenes* between 1 October 2000 and 31 January 2001 and control subjects who were matched with case patients on the basis of ethnicity, sex, age, and pregnancy status. All participants were residents of Winston-Salem.

**Results.** We identified 13 patients, all of whom were Hispanic, including 12 females who were 18–38 years of age. Eleven case patients were pregnant; infection with *L. monocytogenes* resulted in 5 stillbirths, 3 premature deliveries, and 3 infected newborns. Case patients were more likely than control subjects to have eaten the following foods: fresh, unlabeled, Mexican-style cheese sold by door-to-door vendors (matched odds ratio [MOR], 17.5; 95% confidence interval [CI], 2.0–152.5); queso fresco, a Mexican-style soft cheese (MOR, 7.3; 95% CI, 1.4–37.5); and hot dogs (MOR, 4.6; 95% CI, 1.1–19.4). *L. monocytogenes* isolates recovered from 10 female case patients, from cheese bought from a door-to-door vendor, from unlabeled cheese from 2 Hispanic markets, and from raw milk from a local dairy had indistinguishable patterns on pulsed-field gel electrophoresis.

**Conclusions.** This outbreak of listeriosis was caused by noncommercial, fresh, Mexican-style cheese made from contaminated raw milk traced to 1 local dairy. We recommend educating Hispanic women about food safety while they are pregnant, enforcing laws that regulate the sale of raw milk and dairy products made by unlicensed manufacturers, making listeriosis a reportable disease in all states, routinely interviewing case patients, and routinely subtyping clinical *L. monocytogenes* isolates.

Each year in the United States, ~2500 persons become seriously ill as a result of *Listeria monocytogenes* infection, and 500 of these persons die of the infection. Listeriosis is the cause of only 0.02% of illnesses due to foodborne disease, but it is the cause of 27.6% of deaths due to foodborne infection [2]. Pregnant women, newborns, and immunocompromised persons

have the highest risk for acquisition of serious disease due to *L. monocytogenes*. Infection that occurs during pregnancy can precipitate stillbirth, miscarriage, premature birth, and infection in newborns. Sixty percent of cases of listeriosis among persons 10–40 years of age occur among pregnant women, and 27% of cases of listeriosis among persons in all age groups occur among pregnant women [3]. The incidence of listeriosis among

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Hispanic females of childbearing age (i.e., girls and women 15–39 years of age) is 11 times higher than that among non-Hispanic females in this age group [4].

Mexican-style soft cheese made from raw (i.e., unpasteurized) milk has been implicated in outbreaks of infections due to various enteric pathogens, including an outbreak of listeriosis in Los Angeles County, California [5], and outbreaks of infection due to *Salmonella* serotype Typhimurium in northern California [6] and Yakima County, Washington [7]. This cheese is a staple of the Latin American diet, particularly among Mexicans. Although pasteurization of milk greatly reduces the risk of pathogens in dairy products, it is often omitted during non-commercial, unregulated production of fresh, nonaged cheese. *L. monocytogenes* does not tend to survive the aging process required for production of aged, hard cheeses. Fresh, Mexican-style soft cheese is sold door to door and in small markets that cater to Latin American consumers in the United States.

The sale of raw milk is illegal in North Carolina. In August 2000, North Carolina agriculture officials sent warning letters to 2 dairies after discovering that the dairies were selling raw milk to Hispanic residents.

Two months later, public health officials were notified of cases of listeriosis that developed in 3 Mexican immigrants within a 2-week period. We initiated an investigation to determine the extent of the outbreak of listeriosis, the risk factors for infection, the food that was contaminated, and measures for the prevention of such an outbreak. To our knowledge, the present report is the first published report of an outbreak of listeriosis associated with homemade, fresh, Mexican-style cheese traced to raw milk from 1 dairy farm.

## METHODS

**Case definition and case finding.** A “case” was defined by illness with onset between 1 October 2000 and 31 January 2001, in association with isolation of *L. monocytogenes* from a normally sterile site. Case patients were identified by active case finding at the 2 hospitals in Forsyth County, North Carolina. Other regional hospitals and laboratories were contacted as well.

**Case-control study.** A study was conducted to determine the risk factors for pregnancy-related *L. monocytogenes* infection among Hispanic women. A “case patient” was defined as a resident of Winston-Salem, North Carolina, who experienced premature birth, stillbirth, or febrile illness in association with isolation of *L. monocytogenes* from a normally sterile site or with placental tissue samples that were found to be positive for *L. monocytogenes* by staining performed using immunohistochemical techniques from 1 October 2000 through 31 January 2001. For cases of neonatal listeriosis, the mother was considered to be the case patient in the case-control study, and the mother-infant pair was counted as a single case patient.

The source population was defined as female Hispanic res-

idents of Winston-Salem. Control subjects were identified from the county’s prenatal care program registry, Baby Love, which provides outreach and home visits for prenatal care, as well as from an office of the Special Supplemental Nutrition Program for Women, Infants, and Children. Control subjects were matched to case patients by age (to within 1 year) and by pregnancy status; if the control subjects were pregnant, they were matched by the trimester of pregnancy. A woman who was 6 months into the postpartum period was matched to control subjects who were 5–7 months into the postpartum period. A mean of 4 control subjects (range, 3–6 control subjects) were matched to each patient.

An immunosuppressed male patient with listeriosis was excluded from the study because of the difficulty of finding matched control subjects. A standardized questionnaire was used to solicit information on patient symptoms, food consumption (covering consumption of 46 food items), and shopping histories during the month before illness developed. Control subjects were asked to provide data for the same dates for which the matched case patients provided data.

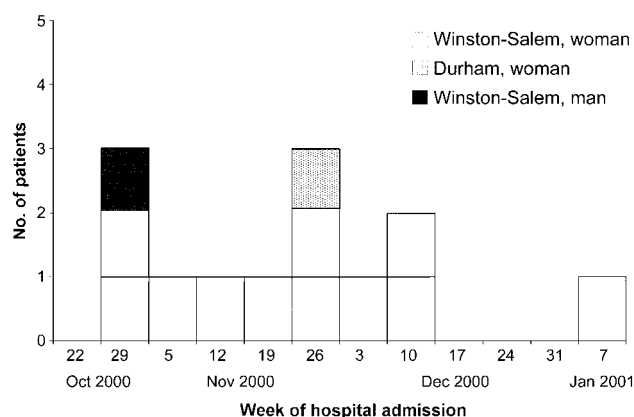
Matched odds ratios (MORs), *P* values, and 95% CIs determined by conditional logistic regression were calculated using SAS software, version 8.0 (SAS Institute). Conditional logistic regression was used for bivariate analyses. The association between covariates was measured using the McNemar test.

**Trace-back investigation.** Interviewers sought to obtain samples of commercial and noncommercial fresh, Mexican-style cheese from the homes of patients. Hispanic stores in Forsyth County were inspected to determine whether they sold noncommercial, fresh, Mexican-style cheese. Interviewers questioned case patients, control subjects, and store owners about individuals involved in the manufacture and distribution of noncommercial, fresh, Mexican-style cheese. Environmental health specialists visited the home of a local cheese maker and obtained environmental samples from the home for culture.

**Dairy inspection and testing.** One manufacturing-grade dairy and 3 grade-A dairies were inspected. (Milk from a manufacturing-grade dairy is used to make such dairy products as cheese and butter. Milk from a grade-A dairy is processed, pasteurized, and sold as a beverage.) Samples of raw milk were obtained from all bulk-milk storage tanks and were tested for the presence of *L. monocytogenes*, and dairy owners were interviewed about sales of raw milk.

The single manufacturing-grade dairy and its cows were inspected again on 8 March 2001, and composite milk samples from each cow were aseptically collected [8]. Somatic cell counts for milk samples obtained from each of the 22 cows were performed using direct microscopy [9]. Milk samples obtained from 7 cows with high somatic cell counts were cultured [10].

**Laboratory investigation.** *L. monocytogenes* isolates recovered from case patients were confirmed using AccuProbe



**Figure 1.** Week of admission of patients with listeriosis to the hospital in North Carolina, from October 2000 through January 2001.

(GenProbe), were serotyped [11], and were subtyped by PFGE performed using *Ascl* and *Apal* restriction endonucleases [12]. Ribotyping was performed using the RiboPrinter microbial characterization system (Qualicon). Also, swab specimens of stool samples obtained from members of the households in which the case patients resided were cultured [13]. Samples of raw milk and of noncommercial, fresh, Mexican-style cheese were cultured using standard methods [14].

## RESULTS

**Case finding and clinical syndrome.** Twelve case patients were identified in Winston-Salem, and 1 patient was identified in Durham, North Carolina, from 1 October 2000 through 31 January 2001 (figure 1). All case patients were Hispanic; 12 were women with a median age of 21 years (age range, 18–38 years), and 1 was a 70-year-old immunocompromised man.

Eleven of the women were pregnant, and, among these women, infection with *L. monocytogenes* resulted in 5 stillbirths, 3 premature deliveries, and 3 infected newborns. The 12th woman was 5 months into the postpartum period when she presented with meningitis caused by *L. monocytogenes*. She had no preexisting medical condition. The male case patient, who presented with a brain abscess, had been receiving corticosteroid therapy since undergoing brain cancer surgery. Symptoms reported by women before admission to the hospital included fever (10 women [83%]), chills (10 women [83%]), headache (10 women [83%]), abdominal cramps (6 women [50%]), stiff neck (6 women [50%]), vomiting (3 women [25%]), and photophobia (3 women [25%]).

**Case-control study.** Eleven case patients and 44 control subjects were enrolled in the case-control study. The case patients were non-English speakers and were Mexican by birth, and they had lived in the United States for a median of 2 years (range, 0–5 years). The control subjects did not differ significantly from the case patients with regard to these characteristics.

According to the results of univariate analyses, case patients, when compared with control subjects, were significantly more likely to have consumed any of the following foods: cheese purchased from door-to-door vendors; queso fresco, a Mexican-style fresh cheese (purchased either from stores or from door-to-door vendors); queso ranchero, another Mexican-style fresh cheese (purchased either from stores or from door-to-door vendors); and hot dogs (table 1). Two variables—queso fresco and queso ranchero—were significantly associated with each other (McNemar test statistic, 11.27;  $P < .05$ ). Consumption of hot dogs was not associated with consumption of queso fresco (McNemar test statistic, 1.50;  $P = .22$ ), queso ranchero (McNemar test statistic, 2.58;  $P = .11$ ), or any cheese bought from a door-to-door vendor (McNemar test statistic, .04;  $P = .83$ ).

**Table 1.** The risk for *Listeria monocytogenes* infection associated with foods consumed by Mexican immigrants in North Carolina from October 2000 through January 2001.

Food	Case patients (n = 11)	Control subjects (n = 44)	Matched OR (95% CI)
Mexican-style cheese			
Bought from a door-to-door vendor	7 (70) <sup>a</sup>	7 (16)	17.5 (2.0–152.5)
Unlabeled	8 (73)	20 (46)	3.6 (0.7–18.4)
Any	9 (82)	30 (68)	2.0 (0.4–11.2)
Queso ranchero <sup>b</sup>			
Any	5 (46)	3 (7)	9.5 (1.8–50.0)
Unlabeled	4 (36)	2 (5)	8.5 (1.5–47.0)
Queso fresco <sup>b</sup>			
Any	8 (73)	13 (30)	7.3 (1.4–37.5)
Unlabeled	7 (64)	7 (16)	8.7 (1.7–44.4)
Queso cuajada <sup>b</sup>			
Any	3 (27)	7 (16)	1.7 (0.4–7.9)
Unlabeled	1 (9)	6 (14)	0.6 (0.1–4.9)
Queso blando <sup>b</sup>			
Any	2 (18)	6 (14)	1.3 (0.3–6.4)
Unlabeled	2 (18)	2 (5)	3.5 (0.5–24.7)
Queso blanco <sup>b</sup>			
Any	4 (36)	16 (36)	1.0 (0.2–4.2)
Unlabeled	4 (36)	11 (25)	1.7 (0.4–6.7)
Queso cotija <sup>b</sup>	1 (9)	7 (16)	0.5 (0.1–4.4)
Hot dog	6 (55)	9 (21)	4.6 (1.1–19.4)
Sliced deli meat			
Turkey	2 (18)	2 (5)	7.5 (0.7–84.2)
Ham	5 (46)	8 (18)	3.5 (0.8–15.4)
Chicken	1 (9)	2 (5)	2.2 (0.1–44.9)
Bologna	0 (0)	2 (5)	0
Cheddar cheese	5 (46)	9 (21)	2.6 (0.7–9.5)
American cheese	3 (30) <sup>a</sup>	16 (36)	0.8 (0.2–3.3)
Mozzarella cheese	1 (9)	17 (39)	0.2 (0.0–1.5)
Blue cheese	0 (0)	2 (5)	0

**NOTE.** Data are no. (%) of case patients or control subjects, unless otherwise indicated.

<sup>a</sup> Denominator is 10 because of missing data.

<sup>b</sup> Type of fresh Mexican-style cheese.

For bivariate analyses, 2 variables—queso ranchero and queso fresco—were combined into 1 new variable. With the new “combined-cheese” variable and the hot dog variable used together in the model, the MOR for hot dog consumption was 7.2, but it did not reach statistical significance (95% CI, 0.9–57.2); however, the MOR for combined-cheese consumption (MOR, 17.8) remained statistically significant (95% CI, 1.9–169.6).

Illness was not associated with the purchase of food from a specific store or with the consumption of raw fruits or vegetables, deli products, other Mexican-style fresh cheeses (including queso cuajada, queso blando, queso blanco, or queso cotija), other types of cheeses (e.g., American, cheddar, mozzarella, blue/Gorgonzola), or other store-bought dairy products (e.g., milk, butter, sour cream, cream, or yogurt). Six of 11 case patients had eaten hot dogs. Two case patients had eaten hot dogs purchased from street vendors in different locations, 3 patients had purchased hot dogs from 3 different supermarket chains, and 1 patient could not recall where she had purchased hot dogs. None could recall the brand names of the hot dogs that were eaten. The case patients were clustered geographically, with 3 neighborhoods or apartment complexes having >1 case patient. No other family member of any of the case patients was reported to have experienced diarrheal illness during the 1 month before the case patients’ onset of illness.

**Trace-back investigation.** *L. monocytogenes* was cultured in noncommercial, fresh, Mexican-style cheese found in the home of 1 patient and in 2 Hispanic grocery stores; it was also cultured in samples of raw milk obtained from the manufacturing-grade dairy. On 16 November 2000, cheese that was culture positive for *L. monocytogenes* was recovered from the home of 1 case patient who reported purchasing noncommercial cheese each week from a woman who came to her door. Case patients reported that noncommercial, fresh, Mexican-style cheese was sold in unlabeled plastic bags at small Hispanic stores, by vendors in various parking lots, and by door-to-door vendors. In November 2000, a few days before becoming ill, the case patient from Durham purchased unlabeled, fresh, Mexican-style soft cheese from a vendor who sold it from a car at a plaza in Mebane, North Carolina, a town located 60 miles from Winston-Salem.

Inspectors found unlabeled homemade cheese in 4 of 15 Hispanic stores that were visited in Forsyth County; the homemade fresh cheese was located next to commercially manufactured, fresh, Mexican-style cheese in refrigerators. Three samples obtained from 2 stores yielded *L. monocytogenes*. Case patients reported purchasing fresh Mexican-style soft cheese from these stores.

Two cheese makers were named by 1 person in the case-control study and by a store owner. Both cheese makers stated that they purchased raw milk from a manufacturing-grade dairy

in Forsyth County. Swab specimens were obtained from the sink drain, buckets, plastic tubs, sink faucet handles, and countertops in the home of 1 cheese maker who sold fresh Mexican-style cheese to a store from which a sample of cheese that was positive for *L. monocytogenes* was obtained. The swab specimens obtained from the household environment were negative for *L. monocytogenes*. This cheese maker reported that she did not pasteurize milk as part of cheese production. Investigators were unable to contact or visit the home of the other cheese maker. Descriptions provided by store owners, case patients, and control subjects indicated that many individuals sell cheese from door to door in this geographic area.

**Dairy inspection and testing.** In Forsyth County, visits were made to a manufacturing-grade dairy and a grade-A dairy during November 2000, and another grade-A dairy was visited in January 2001. The grade-A dairy in Stokes County, North Carolina, was visited in January 2001. One owner of a grade-A dairy in Forsyth County reported selling raw milk to unlicensed individuals (even after being warned by a regional milk specialist from the state’s Dairy and Food Protection Branch in August 2000 that this activity was illegal and posed health risks), as did the manufacturing-grade dairy. The other dairy owners denied selling raw milk to individuals.

Cultures of raw milk samples obtained from the bulk-milk storage tank at the manufacturing-grade dairy yielded *L. monocytogenes* in November 2000 and December 2000 and twice in February 2001. The milk was found to be free of *L. monocytogenes* when samples were obtained on 18 March 2001, after the implementation of revised milking procedures that focused on proper preparation of cow teats and thorough cleaning of equipment. Samples of raw milk obtained from bulk-milk storage tanks at the other dairies were found to be negative for *L. monocytogenes*.

**Laboratory investigation.** *L. monocytogenes* isolates recovered from 10 female patients, cheese samples obtained from 2 stores, cheese samples retrieved from the home of a case patient, and samples of raw milk obtained from 1 local manufacturing-grade dairy were found to be of serotype 4b and ribotype DUP-1042, and they were found to have indistinguishable PFGE patterns by use of *Ascl* (GX6A16.0220) and *Apal* (GX6A12.0059) restriction endonucleases; these findings indicate that the samples that were obtained all shared a common association. None of the 9 stool samples obtained from members of the households in which case patients resided tested positive for *L. monocytogenes*.

## DISCUSSION

We conclude that an outbreak of listeriosis that occurred among recent immigrants from Mexico was caused by consumption of noncommercial, homemade, Mexican-style cheese produced from contaminated raw milk sold to unlicensed cheese makers

by a local dairy. The source of the *L. monocytogenes* at the dairy farm was not identified beyond the bulk-milk tanks. Samples of raw milk obtained from the bulk-milk tanks were found to contain *L. monocytogenes* on numerous occasions, but, after alterations in milking procedures were made, testing of additional samples of raw milk obtained from the bulk-milk tank showed that these samples were free of *L. monocytogenes*.

The PFGE pattern combination of the strain that caused this outbreak is rare. The PulseNet database of the Centers of Disease Control and Prevention (Atlanta, GA) contains PFGE patterns from >2000 human *L. monocytogenes* isolates; among these isolates, this pattern was only identified once in Rhode Island (in 1996), 5 times in Maryland (in 1995, 2000, and 2001) and once in Ohio (in 2000).

To the best of our knowledge, this is the first report of a listeriosis outbreak in the United States that is associated with homemade, fresh, Mexican-style cheese traced to raw milk from a dairy farm. In 1985, an outbreak of listeriosis that involved 86 case patients (including 58 mother-infant pairs) occurred in California. Commercially produced Mexican-style cheese was implicated, and inadequate pasteurization of contaminated raw milk was suspected [5].

The findings of the case-control study are subject to some potential selection and information bias. Efforts were made to select control subjects from the same population from which the case patients were selected. The majority of control subjects came from a county registry for a free prenatal care program that does not require any documentation to obtain services and that actively finds and enrolls patients. We did not record the number of case patients who were enrolled in this registry, to ensure that the source for the control subjects represented the population. Case patients may have had better recall of potential exposures than did control subjects. During the study, rumors spread in the community that the suspected vehicle of infection was homemade Mexican-style cheese.

Misclassification of exposure to special types of Mexican-style fresh cheeses may have occurred. Such cheeses are similar in appearance, texture, and taste, and different names may have been used to describe the same cheese. Moreover, “queso fresco” may be an umbrella term used to describe many of the cheeses that were asked about in the questionnaire.

Contaminated hot dogs have been implicated in previous outbreaks of listeriosis [15–17]. Hot dogs were significantly associated with illness in the outbreak assessed in the present study, according to univariate analyses. However, in bivariate conditional regression models with a combined-cheese variable, hot dogs were not significantly associated with illness. Consumption of hot dogs was not individually associated with consumption of queso fresco, queso ranchero, or any cheese bought from a door-to-door vendor. The epidemiologic data and the molecular subtyping results strongly indicate that infections due

to *L. monocytogenes* were associated with raw milk that originated from the manufacturing-grade dairy.

In Forsyth County, where Winston-Salem is located, an 8-fold increase in the Hispanic population occurred between 1990 and 2000. In the 2000 census, the 16,043 Hispanic residents in Winston-Salem represented 8.6% of the city population; of those residents, 3774 were women of childbearing age. Most Hispanic immigrants in Winston-Salem were born in Mexico (74.2% of Hispanic immigrants in the 2000 census). By dividing the number of female patients in the present study ( $n = 11$ ) by the number of Hispanic women of childbearing age (15–39 years) in Winston-Salem ( $n = 3774$ , according to the 2000 census), we can estimate that the incidence of listeriosis during the outbreak would be at least 291 cases per 100,000 women of childbearing age. If we assume that many women in this age group were not pregnant during the outbreak, it is likely that the incidence of listeriosis was far higher among the subgroup of pregnant Hispanic women during the outbreak. Likely underestimation of this population of Hispanic women of childbearing age in the census may increase the denominator used in the calculation of the incidence of listeriosis and, therefore, may decrease the incidence. In 2001, the overall incidence of infection due to *L. monocytogenes*, according to the United States Foodborne Diseases Active Surveillance Network (FoodNet), was 0.3 cases per 100,000 persons [18].

Large-scale, illegal importation of noncommercial cheeses from Latin America is part of ongoing illegal commerce. During March and April 2002, the US Food and Drug Administration and US Customs began retaining 272–1451 kg of cheese/day at the Hartsfield Airport in Atlanta, Georgia (F. Flores, US Food and Drug Administration, personal communication), indicating a potentially large risk to Hispanic residents of this country.

In 1999, the Council of State and Territorial Epidemiologists recommended that listeriosis be added to the list of nationally notifiable diseases. Listeriosis has been a nationally notifiable disease since 2001. After the occurrence of the outbreak reported in the present study, listeriosis became a reportable disease in North Carolina on 1 June 2001.

The present investigation illustrates many of the challenges associated with dissemination of public health–related information to a new immigrant population. Most case patients and control subjects were recent immigrants, spoke no English, and had no regular health care. Many were socially isolated. The fundamental cause of the outbreak, however, was that many recent immigrants were accustomed to consuming fresh homemade cheese in their home countries and may have been unaware of the associated health risks.

For Hispanic women, we recommend targeted education and dietary counseling about the hazards of eating fresh cheese, undercooked hot dogs, deli meats, and other ready-to-eat meat products implicated as vehicles for listeriosis during pregnancy.

A combination of outreach and enforcement should be directed at store owners, vendors, and dairy farmers, including education about disease risks and vigorous enforcement of laws and regulations governing the production and sale of milk and cheese. Any individuals who have been given a diagnosis of listeriosis should undergo routine interviews about food history conducted by health department staff. All 50 state public health laboratories should solicit and routinely subtype *L. monocytogenes* isolates, in accordance with the protocols of PulseNet, the national molecular subtyping network of public health and food safety regulatory laboratories, to enhance detection and control of similar outbreaks. Clinical laboratory testing of patient isolates may increase the number of cases diagnosed and the recognition of outbreaks of listeriosis.

The outbreak of listeriosis discussed here may represent the tip of the iceberg in terms of adverse health events associated with illegally manufactured, fresh, Mexican-style cheese in the United States. This outbreak may also be a sentinel event for a similar problem associated with listeriosis among pregnant women in Mexico and other Latin American countries, where eating fresh cheese made from unpasteurized milk is prevalent and where detection of listeriosis is hampered by lack of diagnosis and reporting.

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